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Morphometric variation among males of *Orphulella punctata* (De Geer, 1773) (Acrididae: Gomphocerinae) from different biomes in Brazil

Ana Catia Santos da Silva[1], Lorena Andrade Nunes[2], Wanessa de Lima Batista[1], Marcos Gonçalves Lhano[1]

1 Universidade Federal do Recôncavo da Bahia (UFRB), Centro de Ciências Agrárias, Ambientais e Biológicas (CCAAB), Programa de Pós-Graduação em Ciências Agrárias. 44380-000. Cruz das Almas, BA, Brazil.
2 Universidade Estadual do Sudoeste da Bahia (UESB), Programa de Pós-Graduação em Enfermagem e Saúde. 45208-091. Jequié, BA, Brazil.

Corresponding author: *Marcos Gonçalves Lhano* (entomology@gmail.com)

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Abstract

The objective of the present study was to examine variation in the shape and size of pronotum, hind femur, and head in the males of *Orphulella punctata* (De Geer, 1773) from three different Brazilian biomes: the Cerrado, the Atlantic Forest, and the Pantanal. A total of 150 specimens were analyzed from three populations. The results of MANOVA indicated significant differences ($p<0.01$) in the shape of the analyzed structures of *O. punctata* from the different biomes. The results of ANOVA demonstrated significant differences ($p<0.05$) in the size of all analyzed structures. Pearson's correlation analyses among the different structures and the environmental variables revealed that the shape of pronotum, hind femur, and head (dorsal view), as well as the size of pronotum and head (dorsal and lateral views) varied with the geographic longitude, while the shape of hind femur and head (dorsal view) showed a significant negative correlation with size. Results indicated that the shape and size of the analyzed structures, in general, were influenced by the geographical variables.

Key words

geometric morphometrics, hind femur, Orthoptera, population analysis, pronotum, shape, size

Introduction

Grasshoppers are characterized by diverse forms and colors, and a range of ecological and economic importance (Sperber et al. 2012) particularly due to their potential for causing damage to agriculture, as certain grasshopper species are phytophagous with defoliating behaviors (Carrano-Moreira 2015). Among such species, *Orphulella punctata* (De Geer) shows a wide geographical distribution ranging from Mexico to Argentina (Cigliano et al. 2018). This species is common in both native and anthropogenic areas and is considered a pest that causes minor damages to agricultural crops in Brazil (Guerra et al. 2012).

Gomphocerine grasshoppers may be identified by characters of their external morphology (Otte 1979). However, in this subfamily as well as in other grasshopper groups, individuals of the same species occupying different geographical locations may exhibit morphological variation in several structures of their external morphology (Pocco et al. 2014). Geometric morphometrics is a technique that has been used widely in studies conducted on morphological variations at the intraspecific level throughout the geographical distribution range of species (Rattanawannee et al. 2012, Zelditch et al. 2012).

Geometric morphometrics is a tool for detecting morphological variation among the organisms through the identification of landmarks and the subsequent evaluation of the relationships between these landmarks and certain other variables, such as environmental or geographical variables (Klingenberg 2013). This technique also allows the detection of significant differences in the shape and size of body structures with the objective of revealing intraspecific and interspecific variation that may be associated with evolutionary and ecological factors (Bower and Piller 2015). Therefore, this technique has become useful for the identification of variation among the populations of a given species (Oleksa and Tofilski 2015).

Geometric morphometrics also enables the analysis of correlations between the form and size of structures and the patterns of distribution of individuals across various geographical areas as well as with the patterns of diversification of their life histories. As morphometric variations among insect populations are generally associated with differences in geographical and environmental variables, this methodology has proved to be very useful in this group of organisms (Nunes et al. 2012, Romero et al. 2014, Prado-Silva et al. 2016).

Although geometric morphometrics has been demonstrated to be effective in analyzing morphological variation in insects, this methodology has been underused in studies of Orthoptera (Song and Wenzel 2008, Song 2009, Bidau et al. 2012, Cisneiros et al. 2012). In the present paper we analyze morphometric variation in males of *O. punctata* belonging to populations collected from three different biomes in Brazil.

Material and methods

Study area.—The specimens were collected from three Brazilian biomes: the Cerrado, the Pantanal, and the Atlantic Forest. Study

sites were located in: Serra da Bodoquena, Bonito, MS (21°15'56"S, 56°42'10"W); Estrada Porto Cercado, located between river Bento Gomes and the Pantanal Advanced Research Base (BAPP) of the Federal University of Mato Grosso, Poconé, MT (16°18'55.01"S, 56°32'33.64"W); and the Fazenda Baixa de Areia, Highway BA-026, Serra da Jiboia, Varzedo, BA (12°57'41.9"S, 39°26'54.9"W) (Fig. 1).

According to the Brazilian Ministry of Environment (MMA), the Cerrado and the Atlantic Forest are considered biomes of great interest as they are rich in biodiversity and contain several endemic species. The Pantanal is characterized by pronounced wet seasons and is the smallest biome in the country, occupying just 1.76% of the total land area (MMA 2017).

Sampling.—In order to perform the morphometric analyses, a total of 150 *O. punctata* males (50 individuals from the Cerrado, 50 from the Atlantic Forest, and 50 from the Pantanal) were included in the study, except for the analysis of femur structure, where only 34 individuals from the Cerrado biome were included. All the specimens were deposited in the Laboratory of Ecology and Taxonomy of Insects (LETI), Biological Sciences Section, Center for Agricultural, Environmental and Biological Sciences (CCAAB), Universidade Federal do Recôncavo da Bahia (UFRB).

Analyses.—The pronotum (left lateral view, Fig. 2A), hind femur (left lateral view, Fig. 2B), and head (left lateral and dorsal views, Fig. 2C, D) were photographed using Zeiss Discovery V20.0 stereo microscope with an attached camera. The TPS series of programs (Rohlf 2006) were used for the analysis of shape and size, and the images were converted in the TPSUtil program (Rohlf 2010). The landmarks and semi-landmarks (Fig. 2) were inserted using the TPSDig2 program (Rohlf 2006). The Cartesian coordinates were subjected to Procrustes superimposition, and Principal Component Analysis (PCA) and discriminant analysis were performed using MorphoJ software (Klingenberg 2011). MANOVA was used for analyzing the differences in the shape of the structures and ANOVA was used for analyzing the differences in the size (centroid size) of the structures. Pearson's correlations were performed in order to evaluate the relationships between the shape and size of the structures and the geographical variables (latitude, longitude, and altitude), using Past software v. 2.16 (Hammer et al. 2001). Additionally, a cluster analysis was performed using the same program by applying the UPGMA method, and the cophenetic correlation coefficient was calculated.

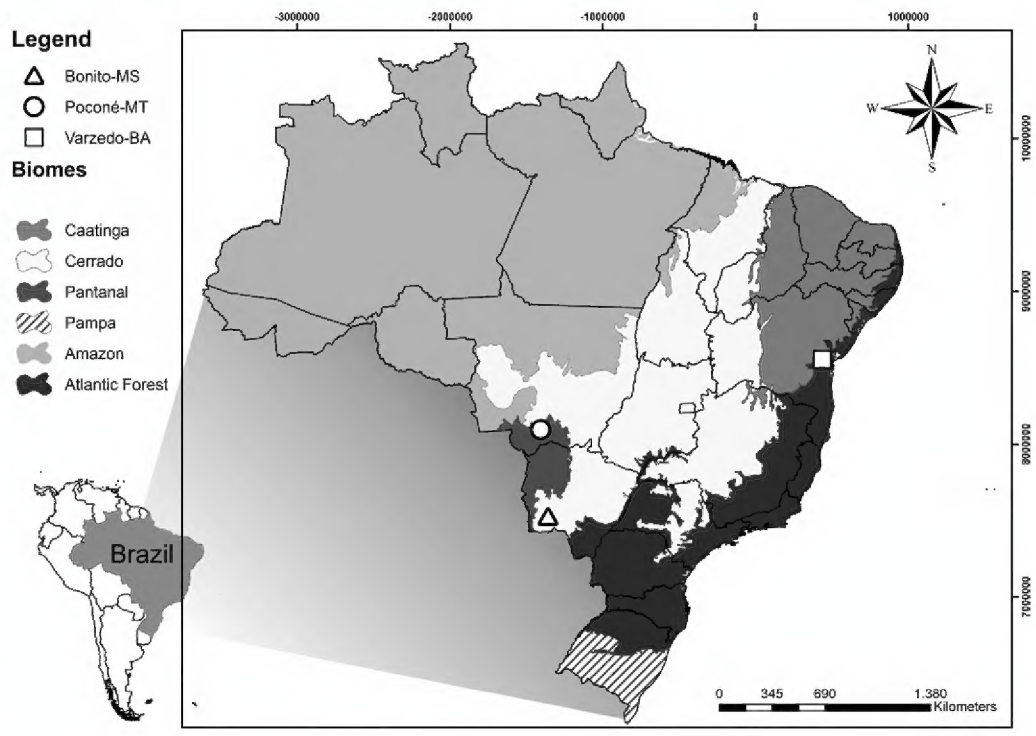

Fig. 1. Collection sites for *Orphulella punctata* (De Geer, 1773): Cerrado, Atlantic Forest and Pantanal.

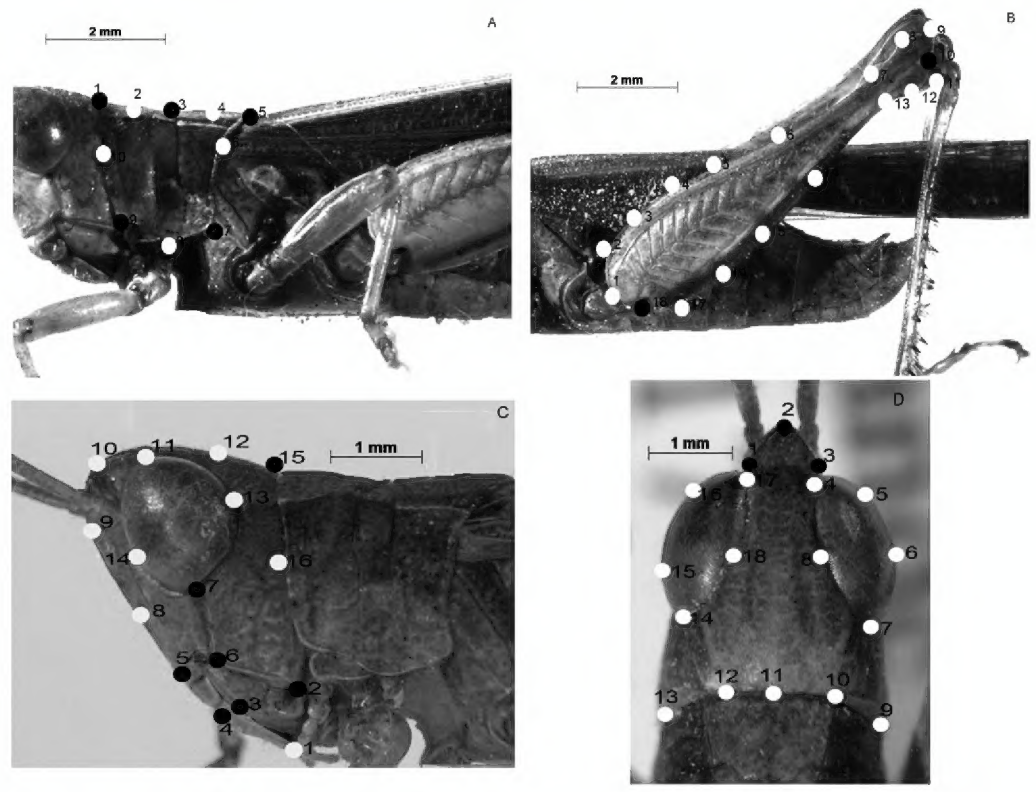

Fig. 2. Lateral view of *Orphulella punctata* (De Geer, 1773). **A.** Pronotum: 10 anatomical points; **B.** Femur: 18 anatomical points; **C.** Lateral view of the head: 16 anatomical points; **D.** Dorsal view of the head: 18 anatomical points. Black circles represent the landmarks and white circles represent the semi-landmarks.

Results

Analysis of pronotum shape in O. punctata.—MANOVA results revealed significant differences ($p<0.01$) in the pronotum shape among the *O. punctata* males from the different biomes that were analyzed. The results of Hotelling's test (T^2) showed that the *O. punctata* males from the Atlantic Forest differed significantly in comparison to the males from the Pantanal and the Cerrado.

The first four components explained 84% of the total variation in the pronotum shape. The first PCA explained 40%, the second one explained 24.8%, the third one 13.6%, and the fourth 5.6%. The greatest variation in the pronotum shape was observed in the dorsal and ventral posterior regions (Fig. 3A, B).

The first PCA also revealed that the specimens from the Pantanal site differed in comparison to the populations from the other biomes (Fig. 3C). Specimens from the Pantanal site were located on the positive axis of PCA 2 and exhibited the greatest distortion in the posterior ventral region of pronotum which expanded, as indicated by the Thin-Plate spline. On the other hand, this structure contracted in the individuals collected from the Cerrado and Atlantic Forest sites (Fig. 3C).

Procrustes distance matrix showed a significant difference ($p<0.01$) in pronotum shape between the three biomes (Table 1).

Table 1. Procrustes distance of *Orphulella punctata* pronotum shape among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0004**	0.0001**
Atlantic Forest	0.0270	0	0.0001**
Pantanal	0.0622	0.0534	0

** significant ($p<0.01$).

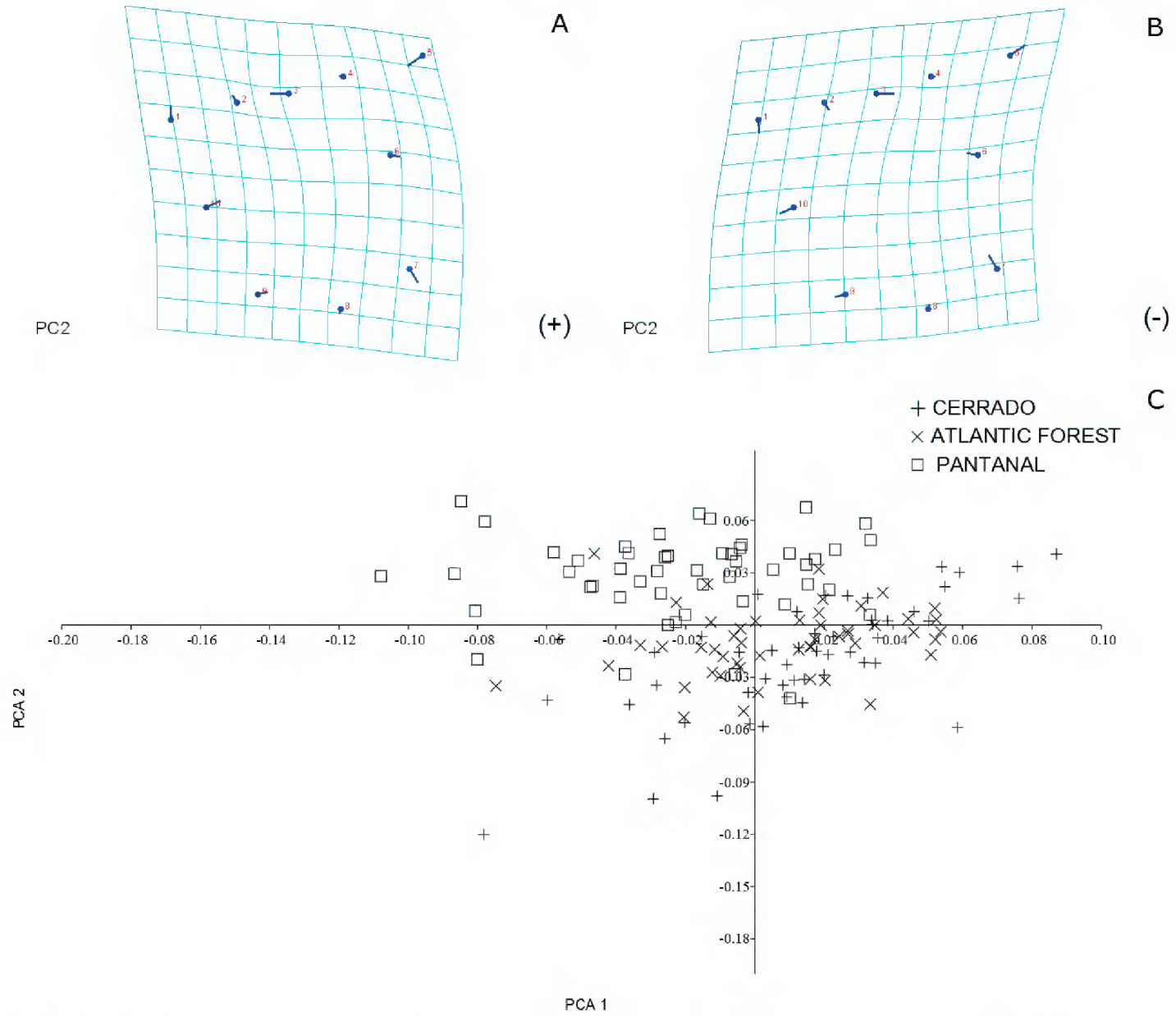

Fig. 3. Scatter plot of the Principal Components Analysis (PCA) of *Orphulella punctata* (De Geer, 1773) pronotum shape in populations collected from the Cerrado, Atlantic Forest, and Pantanal. **A.** Thin-plate spline of the positive (+) and **B.** negative (-) axes of PCA 2; **C.** PCA plot.

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 95% corroborated the above-mentioned results of PCA, demonstrating that the population from the Pantanal was distantly related to the group that was constituted by the populations from the Atlantic Forest and the Cerrado (Fig. 4).

Analysis of the shape of the hind femur.—MANOVA results demonstrated significant differences in the shape of hind femur among the populations from the different biomes ($p<0.01$). The first four PCAs accounted for 78.5% of the total variation in femur shape: the first PCA explained 54.8%, the second one explained 13.3%, the third one 6.1%, and the fourth 4.3%. It was possible to verify differences in the femoral structure among the *O. punctata* populations from the analyzed biomes where specimens from the Cerrado biome were separated from individuals from the other two biomes.

On the positive axis of PCA 1, a contraction in the proximal region (points 2 and 17) of the femur in the individuals from the Pantanal and an expansion in the medial region (points 5, 6, 14, and 15) could be observed (Fig. 5A, C). The inverse of this occurred on the negative axis, i.e. an expansion in the proximal region and a contraction in the medial region of the femur (Fig. 5B).

The Procrustes distance matrix revealed significant differences in shape of the hind femur ($p<0.01$) among the studied populations (Table 2).

Similar to the PCA plot (Fig. 5C), UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 100% showed that the population from the Cerrado biome was distantly

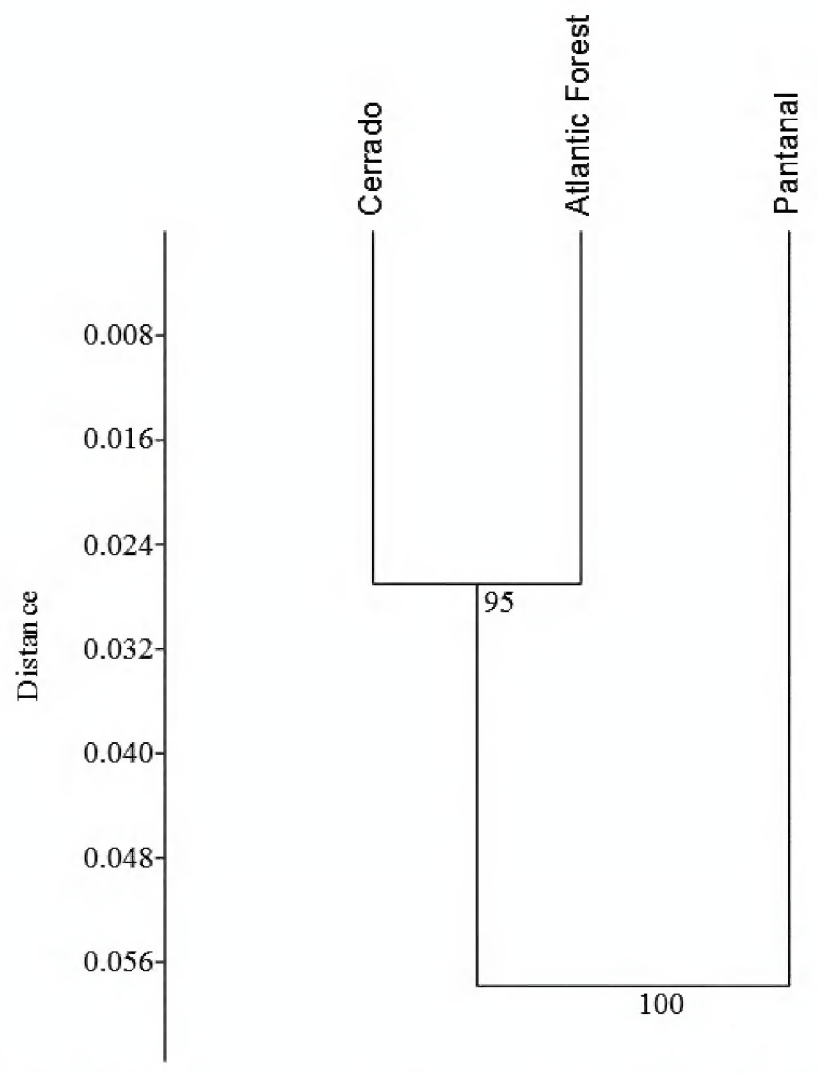

Fig. 4. Similarity dendrogram for the pronotum in *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 97.1%.

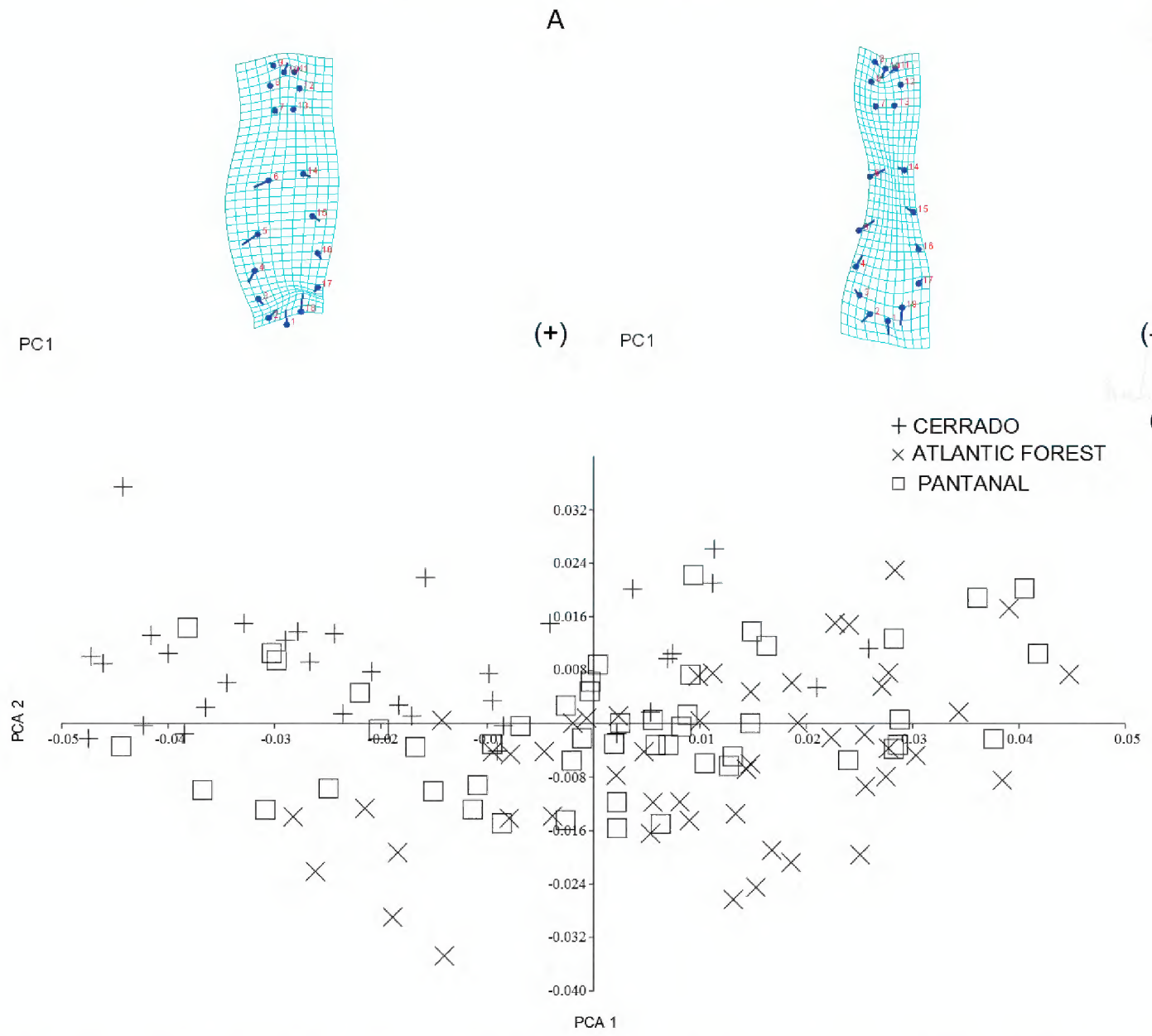

Fig. 5. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) femur shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. **A.** Thin-plate spline of the positive (+) and **B.** negative (-) axes of PCA 1; **C.** PCA plot.

Table 2. Procrustes distance of *Orphulella punctata* femur shape among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0001**	0.0001**
Atlantic Forest	0.0321	0	0.0073**
Pantanal	0.0218	0.0113	0

** significant (p<0.01).

related to the group constituted by the populations from the Atlantic Forest and the Pantanal (Fig. 6).

Analysis of the shape of the head in dorsal view.—MANOVA results revealed significant differences in the shape of head in dorsal view among the *O. punctata* populations from the different biomes (p<0.01). The PCA showed that the first four components explained 77.6% of the total variation: the first PCA explained 44.9%, the second one explained 13.9%, the third one 11.3%, and the fourth 7.5%. The major variations in the shape of head in dorsal view occurred in the distal region of the head (near the pronotum), in the medial portion, and in the fastigium. The greatest distortion occurred in the anterior and medial regions of the head, which expanded on the positive axis, as indicated in the deformation grids (Fig. 7A), and contracted on the negative axis (Fig. 7B).

The second axis of the PCA revealed that the Cerrado population was slightly distinct from the populations of the other biomes (Fig. 7C).

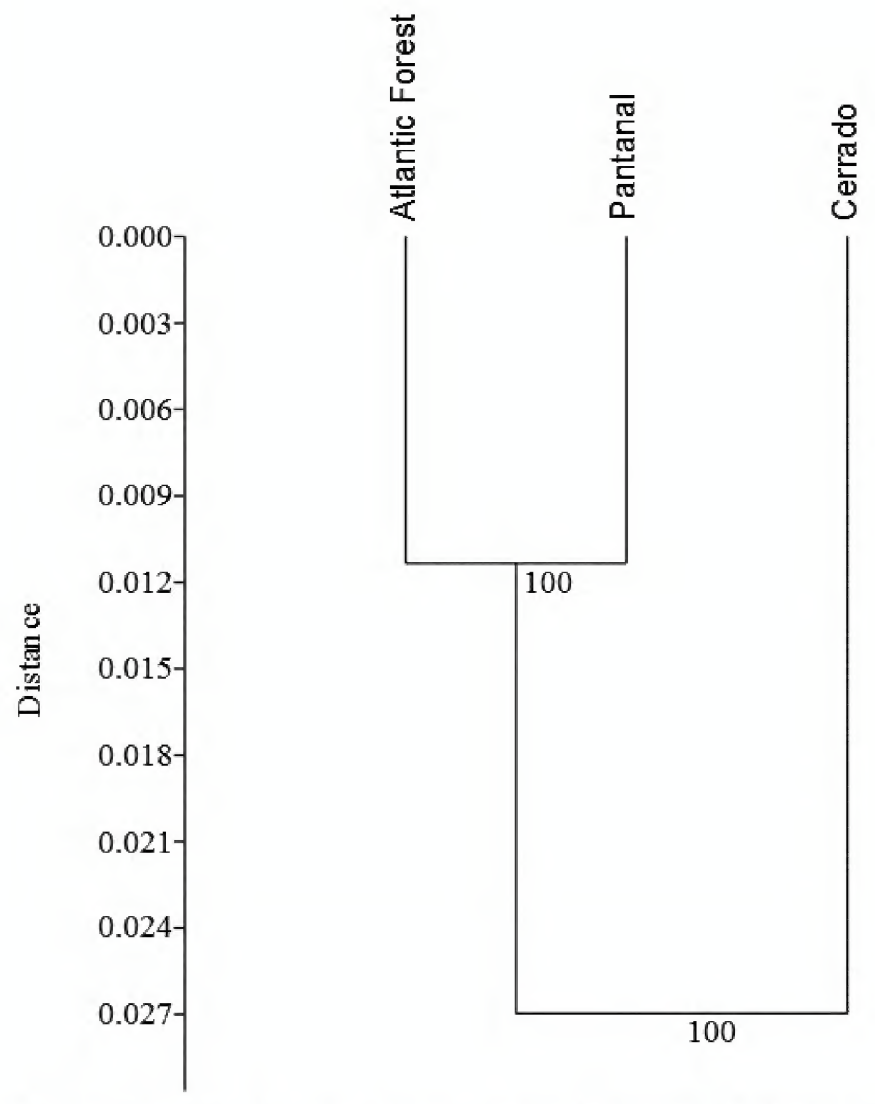

Fig. 6. Similarity dendrogram for the femur from *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 86.83%.

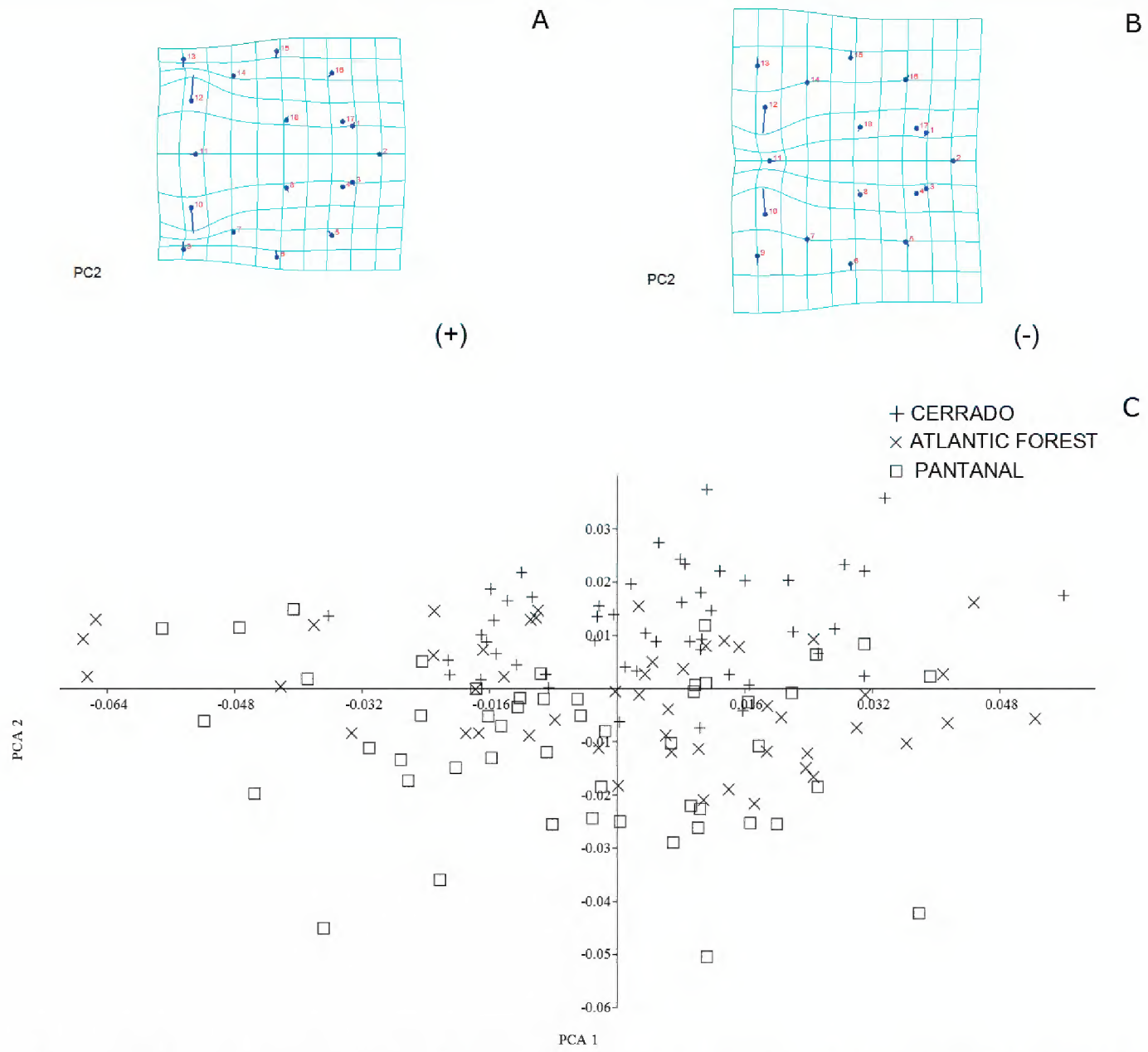

Fig. 7. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) dorsal head shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. **A.** Thin-plate spline of the positive (+) and **B.** negative (-) axes of PCA 2; **C.** PCA plot.

Results from the Procrustes distance matrix demonstrated significant differences ($p<0.01$) in shape of the head in dorsal view among the populations (Table 3).

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 97% corroborated the PCA results, showing that the population from the Cerrado biome was distantly related to the group constituted by the populations from the Atlantic Forest and the Pantanal (Fig. 8).

Analysis of the shape of the head in lateral view.—MANOVA results indicated that the shape of the head in lateral view differed significantly among the *O. punctata* populations from the different biomes ($p<0.01$). The PCA demonstrated that the first four components explained 67.6% of the total variation in this trait. The first principal component explained 28.5%, the second one explained 20.6%, the third one 12.1%, and the fourth 6.4%. The greatest variation in the shape of the head in lateral view was

Table 3. Procrustes distance of *Orphulella punctata* shape of the dorsal view of the head among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0001**	0.0001**
Atlantic Forest	0.0187	0	0.0115*
Pantanal	0.0262	0.0141	0

* significant ($p<0.05$), ** significant ($p<0.01$).

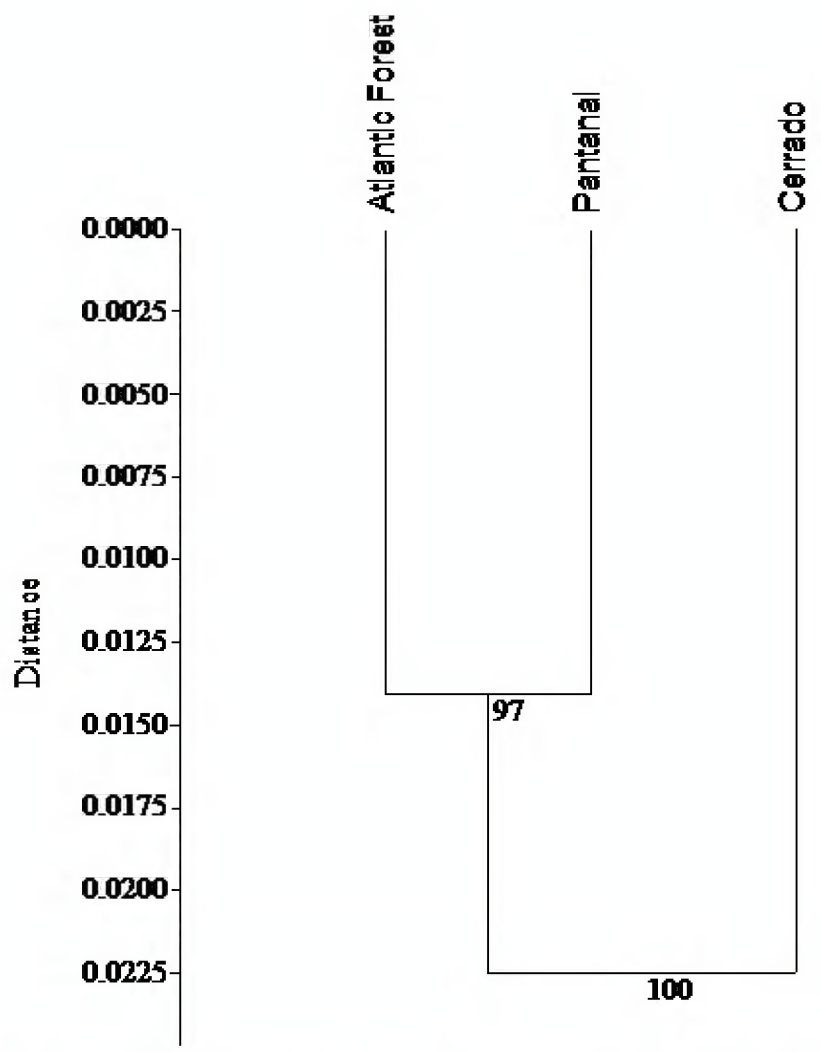

Fig. 8. Similarity dendrogram for the head in dorsal view for *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 79.2%.

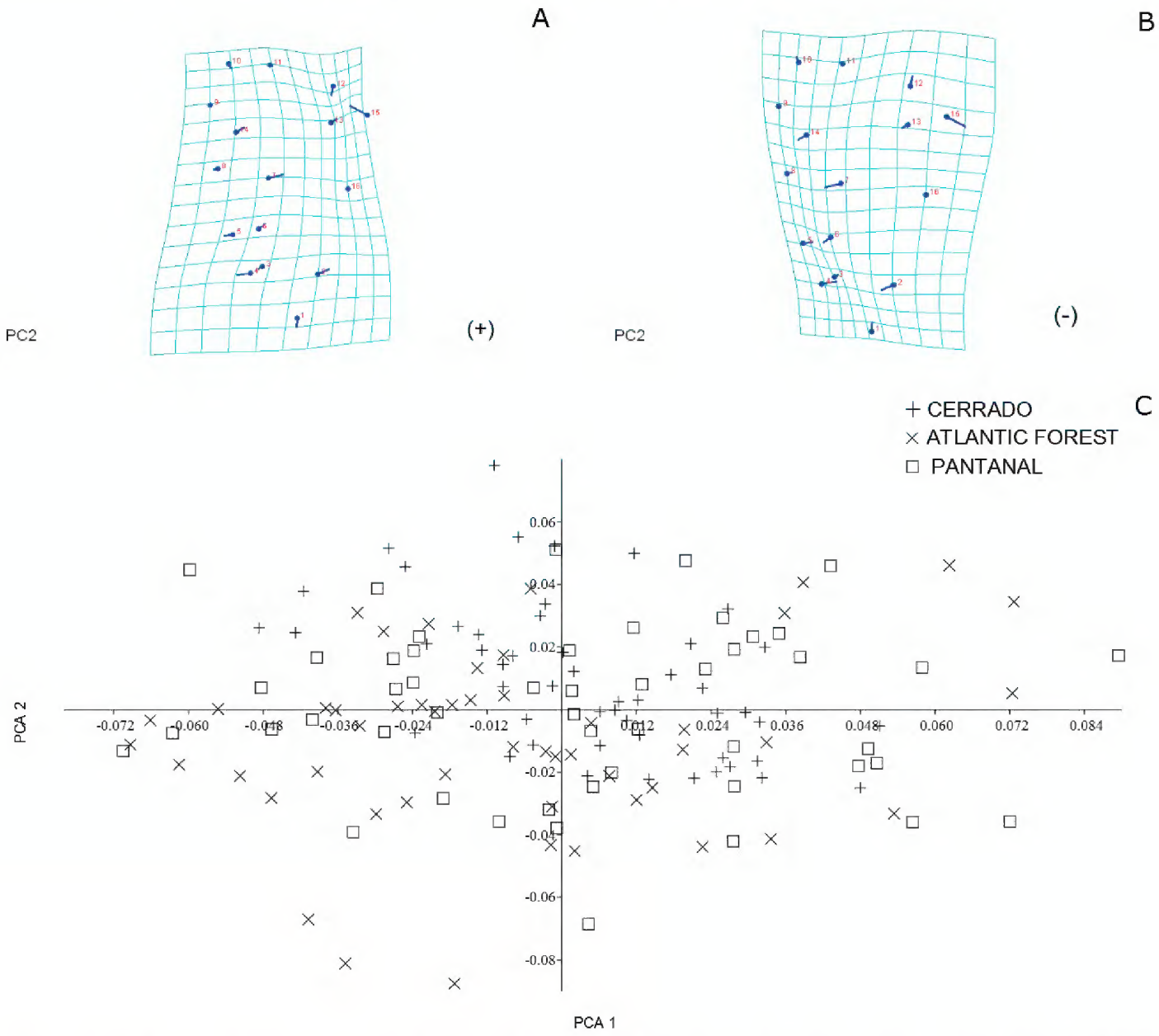

Fig. 9. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) lateral head shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. **A.** Thin-plate spline of the positive (+) and **B.** negative (-) axes of PCA 2; **C.** PCA plot.

observed near the occipital suture and in the eye. Although a uniform distribution occurred, the major distortion was observed in the regions just mentioned, which contracted on the positive axis (Fig. 9A) and expanded on the negative axis (Fig. 9B). The second axis of the PCA did not represent sufficient differences to separate the populations from the different biomes (Fig. 9C).

The Procrustes distance matrix revealed significant differences in the characteristics analyzed ($p<0.01$), indicating differences in the shape of head in lateral view among the populations (Table 4).

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 92% showed that the population from the Cerrado biome formed an independent branch in the dendrogram separated from the group constituted by the Pantanal and the Atlantic Forest populations (Fig. 10).

Analysis of the size of pronotum, hind femur, and head in Orphulella punctata.—ANOVA analyses demonstrated significant differences in size among the populations from the different biomes ($p<0.01$)

Table 4. Procrustes distance of *Orphulella punctata* shape of the lateral view of the head among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0001**	0.0001**
Atlantic forest	0.0360	0	0.0013**
Pantanal	0.0343	0.0257	0

** significant ($p<0.01$).

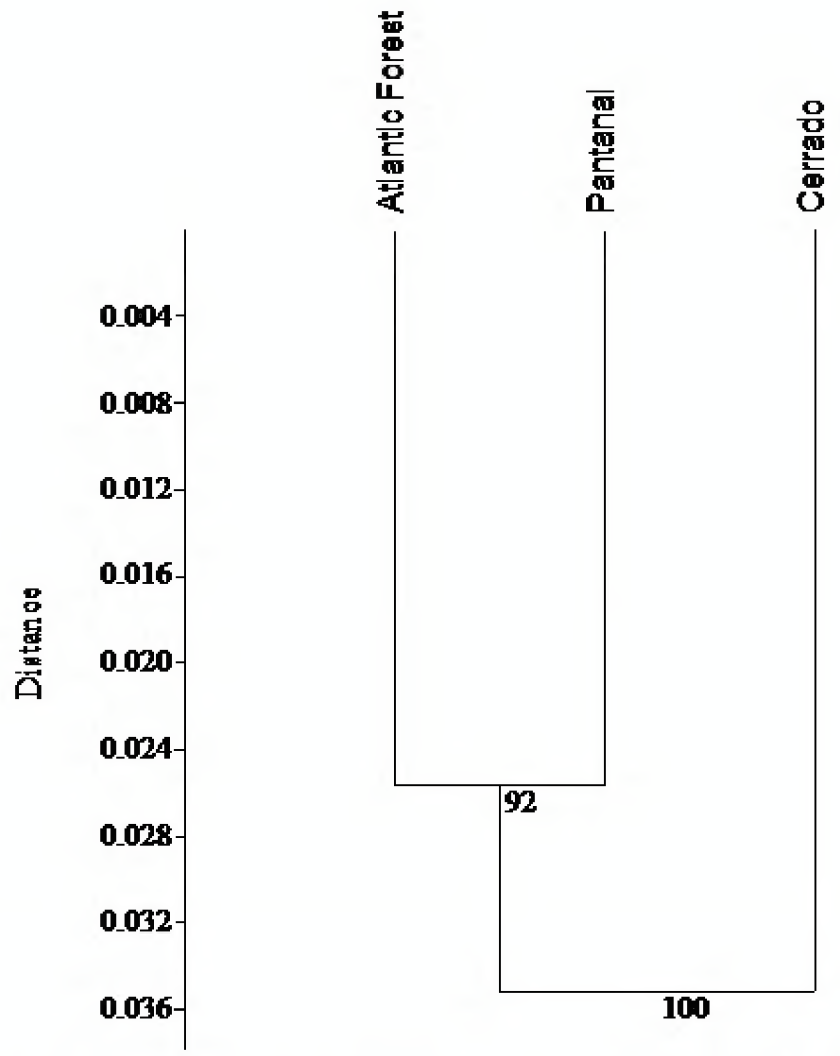

Fig. 10. Similarity dendrogram for the lateral view of the head in *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 98.8%.

for all the analyzed structures. Tukey's test confirmed that pronotum and head in lateral view were larger in individuals from the Cerrado (Fig. 11A, D). The Atlantic Forest population had a larger hind femur in comparison to individuals from the other biomes (Fig. 11B), and the largest size of the head in dorsal view was found in the Pantanal (Fig. 11C).

Correlation analyses between the shape and size of body structures and the geographical variables.—Pearson's correlation analyses between the shape and size of the pronotum, hind femur, and head in *O. punctata*, and the geographical variables (latitude, longitude, and altitude) showed a significant negative correlation between shape × longitude, size × latitude, and size × longitude of the pronotum. A negative correlation was observed between shape × latitude, shape × longitude, and shape × size of the hind femur. The head in dorsal view revealed a negative correlation between size × longitude and shape × size; while the head in lateral view showed a negative correlation between shape × longitude, size × latitude, and size × longitude (Table 5). These results indicated that the morphology of pronotum, hind femur, and head varied in relation to the geographical variables.

Discussion

Among the evaluated structures in *O. punctata*, the hind femur and the head in the population from the Cerrado biome differed in comparison to the other populations from the remaining two biomes. The pronotum of the Pantanal population was significantly different from the other two populations. Nunes et al. (2007), while studying *Melipona scutellaris* Latreille (Hymenoptera: Apidae), suggested that variation among geographically isolated populations might occur due to the existence of a barrier that prevents frequent migrations of individuals. For example, in bees, wing shape is influenced by geographical distance (Nunes et al. 2012, Lima et al. 2014, Prado-Silva et al. 2016). In a previous study, the relationship between shape and geographical distance was highlighted, suggesting that individuals distributed across different latitudes, longitudes, and altitudes might present differences that are associated with adaptations to local conditions (Berner et al. 2004). Similar patterns were observed in the present study, where the correlation analyses indicated that both the shape and the size of the morphological structures analyzed herein might be influenced by the geographical variables.

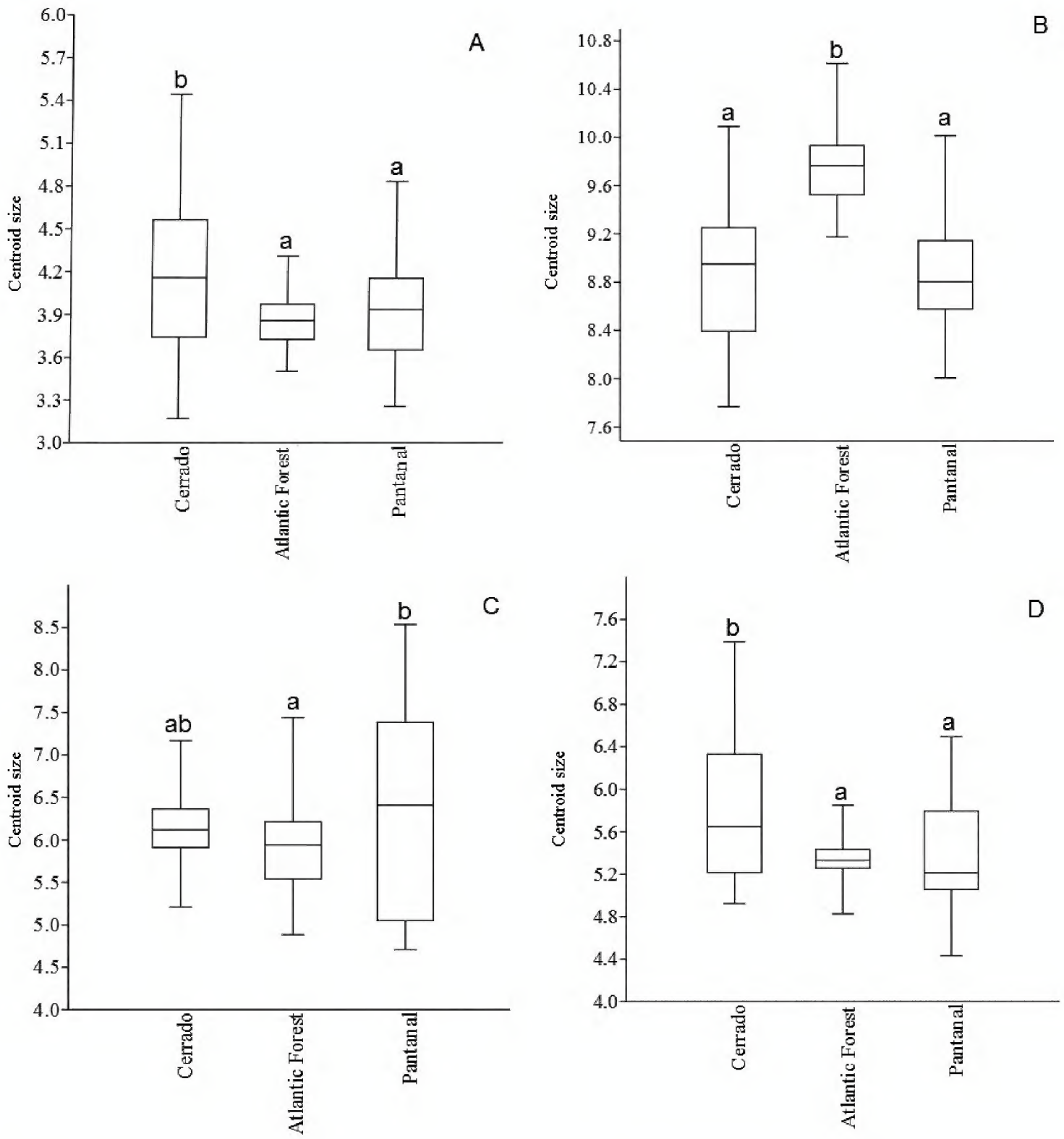

Fig. 11. Analysis of the size of the **A.** pronotum, **B.** femur, **C.** head in dorsal view and **D.** head in lateral view. Similar letters indicate that these biomes are statistically equivalent in relation to the size of the pronotum, femur, dorsal, and lateral view of the head by Tukey's test ($p<0.05$).

Table 5. Correlation between the shape and size of the pronotum, femur, and dorsal and lateral views of the head of *Orphulella punctata* with latitude, longitude, and altitude.

		R	P
Pronotum	Shape × Latitude	-0.0968	0.2383^{ns}
	Shape × Longitude	-0.2295	0.0047**
	Shape × Altitude	-0.1091	0.1837^{ns}
	Size × Latitude	-0.3044	0.0001**
	Size × Longitude	-0.2368	0.0035**
	Size × Altitude	0.2308	0.0044**
	Shape × Size	-0.1516	0.0639^{ns}
Femur	Shape × Latitude	-0.6112	0.0000**
	Shape × Longitude	-0.4583	0.0000**
	Shape × Altitude	0.4414	0.0000**
	Size × Latitude	0.5311	0.0000**
	Size × Longitude	0.6829	0.0000**
	Size × Altitude	-0.0160	0.8544^{ns}
	Shape × Size	-0.4916	0.0000**
Dorsal Head	Shape × Latitude	0.0681	0.4075^{ns}
	Shape × Longitude	-0.0316	0.7007^{ns}
	Shape × Altitude	-0.1518	0.0639^{ns}
	Size × Latitude	-0.0934	0.2551^{ns}
	Size × Longitude	-0.1876	0.0214*
	Size × Altitude	-0.0651	0.4281^{ns}
	Shape × Size	-0.2587	0.0013**
Lateral Head	Shape × Latitude	-0.1412	0.0847^{ns}
	Shape × Longitude	-0.1590	0.0518*
	Shape × Altitude	0.0488	0.5526^{ns}
	Size × Latitude	-0.3526	0.0000**
	Size × Longitude	-0.2239	0.0058**
	Size × Altitude	0.3272	0.0000**
	Shape × Size	0.0080	0.9225^{ns}

* significant ($p<0.05$), ** significant ($p<0.01$), ^{ns} not significant.

Intraspecific variation may complicate taxonomic studies, especially when the variation occurs in the structures that have been used as taxonomic characters for identification, as is the case of the pronotum and head (fastigium) in *O. punctata* (Otte 1979). Intraspecific variation may also occur in the internal structures in grasshoppers, as demonstrated by Song and Wenzel (2008) in a study on the male genitalia morphology among three populations of *Schistocerca lineata* Scudder.

Species that exhibit a wide geographical distribution tend to present both geographical variations and polymorphism, which may lead to incorrect identifications (Pocco et al. 2014). *O. punctata* is widely distributed from Mexico to Argentina, with polymorphism as a common observation among its populations (Dixon 2005). Liebermann (1947) considered that *O. punctata* may be one of the acridid species with greatest variation in size and color. Rehn (1906) was the first to report the existence of variation in the structure and color among individuals belonging to different populations collected from diverse regions of Brazil and the U.S. Virgin Islands. In 1916, the same author (Rehn 1916) remarked on the existence of a number of variations among the populations of this plastic and widespread species collected from three different northern states of Brazil. Rehn (1918) then reported the existence of variation in the head and pronotum of the individuals belonging to two populations from Para, Brazil. Bruner (1913) also reported the existence of variation in size among the individuals of *O. punctata* collected from Peru.

Hebard (1923) described a remarkable variability of this species, especially in size, pronotal contour, and coloration, which has often led to misidentifications generating unusually complex synonymies (including the description of *O. punctata* populations as different species placed in different genera such as *Oxycoryphus* and *Stenobothrus*). Cigliano et al. (2018) reported 19 synonyms of *O. punctata*.

In a taxonomic review of this group, Otte (1979) considered that *O. punctata* is easily misidentified with *O. aculeata* Rehn in central Mexico, and with *O. losamatensis* Caudell and *O. concinnula* (Walker) in Central and South America. The same author nonetheless mentioned the importance of the hind femur and shape of the pronotum as the characteristics for distinguishing *O. punctata* from the remaining species of *Orphulella*. However, and according to the results obtained in the present study, the hind femur and pronotum also displayed variations among the populations from different biomes, and hence other structures and/or methods of identification are required for the purpose of a correct identification of this species.

In addition to the structures suggested by Otte (1979) as diagnostic characters for identification of *O. punctata*, the present study showed that the head also presented intraspecific variation, reinforcing the needs for other structures to be used in the identification of this species. Intraspecific variations in the morphological characters, such as head, pronotum, femur, body, and wings, have also been reported in studies conducted on the populations of *Chromacris speciosa* (Thunberg) collected from two locations in Pernambuco, Brazil (Cisneiros et al. 2012). Also, analyses of the body size and the wings of *Trilophidia annulata* (Thunberg) (Orthoptera: Oedipodinae) demonstrated significant differences among the populations collected from different kinds of environments in China (Bai et al. 2016), which corroborates the occurrence of variations in individuals of the same species living in different biomes. Whitman (2008) demonstrated that body size in Orthoptera varies both between and within species, mainly as a result of environmental factors.

It is concluded that the shape and the size of the analyzed structures of *O. punctata* vary among biomes, indicating the possible influence of environmental conditions on the variations in the morphology of this species. The geometric morphometrics analyses conducted in this study indicated that it is possible to separate the populations from different biomes by the shape and size of their various body parts.

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